

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**

THIS PAGE BLANK (USPTO)

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

A12

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12Q 1/68	A1	(11) International Publication Number: WO 99/09212 (43) International Publication Date: 25 February 1999 (25.02.99)
(21) International Application Number: PCT/GB98/02461 (22) International Filing Date: 18 August 1998 (18.08.98) (30) Priority Data: 9717458.5 18 August 1997 (18.08.97) GB (71) Applicant (for all designated States except US): QUEEN MARY & WESTFIELD COLLEGE [GB/GB]; Mile End Road, London E1 4NS (GB). (72) Inventors; and (75) Inventors/Applicants (for US only): NEWLAND, Adrian, Charles [GB/GB]; Queen Mary & Westfield College, Mile End Road, London E1 4NS (GB). JIANG, Xurong [CN/GB]; Queen Mary & Westfield College, Mile End Road, London E1 4NS (GB). (74) Agents: SHEARD, Andrew, Gregory et al.; Kilburn & Strode, 20 Red Lion Street, London WC1R 4PJ (GB).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: TELOMERASE ASSAY (57) Abstract An assay for the detection of telomerase which consists of contacting the sample with a telomerase substrate primer and with oligonucleotides such that extension products are formed in the presence of telomerase, amplifying the extension products by PCR and detecting the PCR products. The telomerase substrate primer is labelled with a fluorescent label and the PCR products are fluorescent and can easily be detected.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

TELOMERASE ASSAY

The present invention relates to an assay for the detection of telomerase and, in particular to a fluorescence based assay.

5

Telomerase is an enzyme that maintains telomeres, the protein-DNA caps covering the ends of chromosomes. Chromosomes lacking telomeres are known to undergo fusions, rearrangements and translocations, are degraded by cellular enzymes and eventually become lost as the cell divides. Telomeres protect chromosomal DNA from such instabilities. When telomerase is absent from a cell, telomeres become shorter with each cell division as a small piece of DNA is lost from the each of the chromosome ends. Because most cells have no telomerase activity, their telomeres shorten with age and the cells eventually stop dividing and exit the cell cycle. Telomere shortening is thought to be a cellular sign of aging. Spermatogonial and ovary cells (germ cells) are an exception. Their chromosome ends are maintained by a high level of telomerase and do not shorten over time.

15

In 1994, Kim *et al* (*Science*, 266, 2011-2015 (1994)) developed a sensitive assay for telomerase known as the telomerase repeat amplification protocol (TRAP) assay and then demonstrated a dramatic correlation between cancer and telomerase. Investigators found high levels of telomerase activity in 90 out of 101 distinct tumours representing 12 cancer types and in 98 out of 100 immortal cell lines. In contrast, the enzyme was not detected in benign tumours or in mortal cell lines. These results suggest that telomerase may be necessary for cells to become immortal and also that it would be a good target for chemotherapy in cancer patients.

20

25

Furthermore, it is generally agreed that telomerase shows promise as a biomarker for early stage cancers and could aid in distinguishing between aggressive and benign tumours.

30

In the TRAP assay developed by Kim *et al*, telomerase synthesises extension products which are then amplified by the polymerase chain reaction (PCR). The assay uses a telomerase substrate primer designated TS and ^{32}P labelled oligonucleotides. Telomerase, if present, synthesises telomeric repeats onto the TS oligonucleotide and these telomerase products are then amplified by PCR using a further primer designated CX.

Although the assay developed by Kim *et al* is relatively sensitive and semi-quantitative, it does have some associated problems. Firstly, it involves the use of ^{32}P -labelled dCTP or dGTP and analysis of radioactive PCR products and secondly, some tumour samples give false negative results due to the presence of *Taq* polymerase inhibitors (Wright *et al*, (1995) *Nucleic Acids Res.*, 18, 3794-3795). This is clearly undesirable if the assay is required to form the basis of a reliable diagnostic tool.

The TRAP assay was modified by Savovsky *et al* (*Nucleic Acids Res.* (1996) 24(6), 1175-1176) by combining it with a scintillation proximity assay which makes use of [methyl ^3H] TTP and thus avoids the analysis of ^{32}P -labelled reaction products. However, radioactivity is still involved and this assay does not solve the problem of the false negatives.

Further improvements to the reliability of the TRAP assay were made by Wright *et al*, (*Nucleic Acids Res.*, (1995) 18, 3794-3795) who introduced an internal standard. However, even using this method, quantification is tedious and not always completely successful.

The present invention relates to an improved assay for the detection of telomerase which makes use of a fluorescent labelled substrate.

In the present invention there is provided a method for the detection of telomerase in a sample, the method comprising:

i. contacting the sample with a fluorescent labelled telomerase substrate oligonucleotide primer and with dATP, dCTP, dGTP and dTTP such that extension products are formed if telomerase is present in the sample;

5

ii. amplifying the extension products, if present, using the polymerase chain reaction (PCR); and

iii. detecting the presence of fluorescent PCR products.

10

The fluorescence based assay of the present invention has several advantages over the prior art. Firstly, because fluorescence is used to detect the presence of amplified telomerase, the use of radioactivity is avoided. Thus the process is considerably less hazardous than assays based on the detection of a radiolabelled product.

15

In addition, the assay of the present invention is about 50 to 100 times more sensitive than the TRAP assay and its various modifications described in the prior art. Indeed; the usual number of cells needed in the TRAP assay to detect telomerase is about 100 cells or more whereas the assay of the present invention is sufficiently sensitive to detect telomerase activity at the single cell level.

20

Thirdly it is relatively simple to use the assay method of this invention to obtain quantitative results since the inventors have found that for small numbers of cells, for example up to fifty cells, there is a correlation between the cell number and the peak area. Thus the method permits telomerase quantification and comparisons between samples.

25

Also, it has been found that, unlike the TRAP assay, no false negatives were obtained using the assay of the present invention. Clearly this is extremely important if the assay is to be used as a diagnostic tool.

30

In the method of the present invention, a typical sample to be assayed will comprise from about 0.5 to 2000 cells, more usually from about 1 to 1000 cells, preferably from 1 to 500 cells and most preferably from about 1 to 100 cells.

5

Thus, the assay of the present invention is considerably more sensitive than the TRAP assay described by Kim *et al* where at least about 100 cells per sample are required for the detection of telomerase and a typical sample contains from about 2000 to 10000 cells.

10

Furthermore, the increased sensitivity of the assay of the present invention has also resulted in the elimination of false negative results which tend to appear when high cell numbers are used and which may result from the presence in the sample of *Taq* polymerase inhibitors.

15

The telomerase substrate primer used in the present invention may be the TS primer described by Kim *et al* which has the sequence:

5'-AATCCGTCGAGCAGAGTT-3'

20

However, in the method of the present invention, the telomerase substrate primer is labelled with a fluorescent marker instead of using radiolabelled nucleotides as in the TRAP assay.

25

A wide range of fluorescent markers is available and suitable choices of marker include, for example, fluorescein dyes such as 6-carboxyfluorescein (6-FAM), 4,7,2',4',5',7'-hexachloro-6-carboxyfluorescein (HEX), or 4,7,2',7'-tetrachloro-6-carboxyfluorescein (TET). Alternatively, the marker may be a rhodamine dye such as N,N,N,N-tetramethyl-6-carboxyrhodamine (TAMRA) or 6-carboxy-X-rhodamine (ROX), or a compound such as JOE, RG6, RG110 or Cy5. Other available fluorescent

30

markers will be familiar to those skilled in the art. The label is preferably attached to the 5' end of the primer.

5 For a typical sample size, the telomerase substrate primer may be present in an amount of from about 0.05 μ M to 1.0 μ M, often about 0.1 μ M. Each deoxyribonucleotide may be present in an amount of from 10 to 100 μ M, more usually from 40 to 60 μ M and generally about 50 μ M.

10 From about 20 to 60 PCR cycles may be used in the amplification step although it is usual to use from about 20 to 40, and typically about 30, cycles.

The PCR primer may be the CX primer employed by Kim *et al* which has the sequence:

15 5'-(CCCTTA)₃CCCTAA-3'.

For a typical sample size, the PCR primer may be present in an amount of 0.05 to 1 μ M, often about 0.1 μ M.

20 The fluorescent PCR products may be detected by any conventional means, for example using a DNA analyser such as the ABI PRISM™ 373/377 DNA Sequencer or the ALFexpress™ DNA Sequencer. As already mentioned, one of the advantages of the method of the present invention is that it enables quantitative measurement of both the size of the amplified telomerase extension products and of the amount of telomerase
25 originally present in the sample.

The telomerase band sizes may be measured by any conventional means, for example by electrophoretic comparison with a ladder of fragments of known sizes. Such ladders are commercially available.

It has been found that the amount of telomerase originally present in the sample is proportional to the peak area of the detected PCR product. The consistency of this proportional relationship is much greater than for prior art methods based on the TRAP assay and, indeed, the present inventors have shown that there is a log-linear relationship between the amount of telomerase before amplification and the total peak area.

The method of the present invention may be carried out with the use of a kit and therefore in a second aspect of the invention there is provided a kit for the detection of telomerase, the kit comprising:

- i. a fluorescent labelled telomerase substrate oligonucleotide primer;
- ii. suitable amounts of dATP, dCTP, dGTP and dTTP; and
- iii. a PCR primer.

It is greatly preferred for the kit of the invention also to contain a DNA ladder which is a fluorescent dye and, in addition, TAQ polymerase and a TAQ buffer. These components are needed in order for the kit to be self sufficient.

The components of the kit will be provided in separate containers.

The telomerase substrate primer may be present in an amount of from about 0.05 μ M to 1.0 μ M, often about 0.1 μ M. Each deoxyribonucleotide may be present in an amount of from 10 to 100 μ M, more usually from 40 to 60 μ M and generally about 50 μ M. The PCR primer may be present in an amount of 0.05 to 1 μ M, often about 0.1 μ M.

Preferred primers are as for the first aspect of the invention.

The invention will now be further described in the following examples, which are not intended to be limiting, and in the accompanying drawings in which:

5

FIGURE 1 shows PCR-based fluorescence-ABI detection of telomerase activity in CEM/VLB₁₀₀ leukaemic cells;

10

FIGURE 2 is a plot showing the log-linear relationship (and its 95% confidence interval) between cell numbers and the sum of peak area for telomerase activity; and

15

FIGURE 3 is a photograph of a gel showing the detection of telomerase activity using the method of the invention in a variety of human myeloid and B-lymphoid leukaemic cell lines.

Example 1

a. Preparation of Cell Extracts

20

Cell extracts from human T-lymphoblastic leukaemia cell line (CEM/VLB₁₀₀) were prepared by the following method based on that of Kim *et al.* Cells were washed once in phosphate buffered saline (PBS), pelleted at 10,000g for 1 minute at 4°C, resuspended in ice cold wash buffer (10mM Hepes-KOH (pH7.5), 1.5mM MgCl₂, 10mM KCl, 1mM dithiothreitol), pelleted again and resuspended at 10⁶ cells per 100µl of ice cold lysis buffer (10mM tris-HCl (pH7.5), 1mM MgCl₂, 1mM EGTA, 0.1mM phenylmethylsulphonyl fluoride, 5mM β-mercaptoethanol, 0.5% CHAPS (Pierce), 10% glycerol). The suspension was incubated for 30 minutes on ice and then centrifuged for 30 minutes in a microultracentrifuge (16,000 x g, 4°C). The supernatant was removed, quick frozen on dry ice and stored at -80°C.

30

b. Extension and Amplification

Assay tubes were prepared by lyophilising 0.1µg of CX primer onto the bottom of the tube and sealing it with 7 to 10µl of molten wax (Ampliwax, Perkin-Elmer). After the wax was allowed to solidify at room temperature, the tubes were stored at 4°C. Five microlitre reactions above the wax barrier contained 10 x *Taq* buffer, 0.01µg 5' 6-FAM labelled TS primer, 0.1µg of T4g32 protein (Boehringer Mannheim), 0.1U of *Taq* DNA polymerase (Boehringer Mannheim) and 50µM deoxynucleoside triphosphates. After 30 min at 25°C, for extension of oligonucleotide TS by telomerase, the tubes were transferred to an oil free thermal cycler for 30 rounds at 94°C for 30 seconds, 50°C for 30 seconds and 72°C for 1.5 minutes.

The PCR products (1µl of each) were analysed using an ABI 373 DNA analyser (Perkin Elmer, USA) running at 2,500 volts, 30 mA and 30 watts for 2 hours. Telomerase band sizes in base pairs were obtained by co-electrophoresis of a fluorescently labelled ladder (Tamra GS350) consisting of DNA fragments of known molecular weight. Base pair sizes were calculated using 672 Genescan software employing a local Southern fit. The software also provides peak height and peak area values for each peak of fluorescence detected. The 1µl aliquot required for this detection system allows the same sample to be analysed several times from a very small PCR reaction volume.

The sensitivity of the detection method is shown in Figure 1. In Figure 1, cells have been titrated from 0.125 to 128 and telomerase activity was represented by the 6-bp random repeats. The figure shows that in this serial dilution, telomerase activity was detected at the single cell level without the need to increase either the incubation time (up to 1 hour) or the number of PCR cycles (up to 34). When diluted down to 0.5 cell, the telomerase activity is still detectable under ABI analyser in CEM/VLB₁₀₀ drug-resistant lymphoblastic leukaemia cells. The method of the invention is at least 50 to 100 times more sensitive than the conventional method of Kim *et al* which requires at

least 100 cells for the detection of telomerase. The specificity was confirmed by treatment of cell lysate with RNAase A which completely diminished the 6-bp repeats signal.

5 The combined peak area of the first six bp peaks was used to quantify the total telomerase activity prior to PCR amplification. Figure 2 demonstrates that there is a log-linear relationship between cell numbers and the sum of peak area for telomerase activity ($r = 0.98$, $p = 0.00001$). This relationship remained the same when up to nine peaks were used to quantify the telomerase activity.

10

When using the standard assay of Kim *et al*, with 6 μ g protein per assay (equivalent to 2,000 to 10,000 cells), some tumour samples have been observed to give false negative results for telomerase due to the presence of *Taq* polymerase inhibitors although testing positive when the samples are diluted.

15

In the assay method of the present invention, where it is possible to use less than 50 cells for analysis, no such false negative results were observed. Also, the correlation between log-cell number and peak area was high for cell numbers between 1 and 128 giving an $r^2 = 0.97$, thus permitting quantitation and comparisons between samples.

20

Example 2

Telomerase Activity of a Variety of Cell Lines

To confirm further the sensitivity and reliability of the fluorescence based assay,
25 Example 1 was repeated using the human myeloid and lymphoid leukaemic cell lines, human lymphoma cell lines shown in Table 1 as well as mouse fibroblast sarcoma cell line and human colon carcinoma cell lines. Single cell preparations were used.

Figure 3 illustrates the results from the cell lines shown in Table 1 which show that
30 telomerase can be both detected and quantified in single cells of all of these cell lines.

Table 1

<u>Cell Line Type</u>	<u>Cell Line</u>
Human myeloid	U937
	HL-60
	KG1A
	K562
T-lymphoid	CCRF-CEM
	CEM/VLB ₁₀₀
	Jurkat
	H9
B-lymphoid	Daudi
	Raji
	Ramos

Thus, the present invention provides a method for the quantitative detection of
5 telomerase activity.

CLAIMS

1. A method for the detection of telomerase in a sample, the method comprising:
 - 5 i. contacting the sample with a fluorescent labelled telomerase substrate oligonucleotide primer and with dATP, dCTP, dGTP and dTTP such that extension products are formed if telomerase is present in the sample;
 - ii. amplifying the extension products, if present, using the polymerase chain
10 reaction (PCR); and
 - iii. detecting the presence of fluorescent PCR products.
2. A method as claimed in claim 1, wherein the sample to be assayed comprises
15 from about 0.5 to 2000 cells.
3. A method as claimed in claim 2, wherein the sample to be assayed comprises from about 1 to 100 cells.
- 20 4. A method as claimed in any one of claims 1 to 3, wherein the telomerase substrate primer has the sequence:

5'-AATCCGTCGAGCAGAGTT-3'.
- 25 5. A method as claimed in any one of claims 1 to 4, wherein the telomerase substrate primer is labelled with a fluorescein dye such as 6-carboxyfluorescein (6-FAM), 4,7,2',4',5',7'-hexachloro-6-carboxyfluorescein (HEX), or 4,7,2',7'-tetrachloro-6-carboxyfluorescein (TET); a rhodamine dye such as N,N,N,N-tetramethyl-6-carboxyrhodamine (TAMRA) or 6-carboxy-X-rhodamine (ROX); or with
30 a compound such as JOE, RG6, RG110 or Cy5.

6. A method as claimed in any one of claims 1 to 5, wherein the fluorescent label is attached to the 5' end of the telomerase substrate primer.

5

7. A method as claimed in any one of claims 1 to 6, wherein the telomerase substrate primer is present in an amount of from about 0.05 μ M to 1.0 μ M.

10

8. A method as claimed in any one of claims 1 to 7, wherein each deoxyribonucleotide is present in an amount of from 10 to 100 μ M.

9. A method as claimed in any one of claims 1 to 8 wherein from about 20 to 40 PCR cycles are used in the amplification step.

15

10. A method as claimed in any one of claims 1 to 9, wherein the PCR primer has the sequence:

5'-(CCCTTA)₃CCCTAA-3'.

20

11. A method as claimed in any one of claims 1 to 10, wherein the PCR primer is present in an amount of 0.05 to 1 μ M.

12. A method as claimed in any one of claims 1 to 11, wherein the fluorescent PCR products are detected using a DNA analyser.

25

13. A method as claimed in any one of claims 1 to 12, wherein the telomerase band sizes are measured by electrophoretic comparison with a ladder of fragments of known sizes.

30

14. A kit for the detection of telomerase, the kit comprising:

- i. a fluorescent labelled telomerase substrate oligonucleotide primer;
- ii. suitable amounts of dATP, dCTP, dGTP and dTTP; and
- iii. a PCR primer.

15. A kit as claimed in claim 14, further comprising:

- iv. A DNA ladder which is a fluorescent dye;
- v. TAQ polymerase; and
- vi. A TAQ buffer.

16. A kit as claimed in claim 14 or claim 15, wherein the telomerase substrate primer is present in an amount of from about 0.05 μ M to 1.0 μ M.

17. A kit as claimed in any one of claims 14 to 16, wherein the telomerase substrate primer has the sequence:

5'-AATCCGTCGAGCAGAGTT-3'.

18. A kit as claimed in any one of claims 14 to 17, wherein the telomerase substrate primer is labelled with a fluorescein dye such as 6-carboxyfluorescein (6-FAM), 4,7,2',4',5',7'-hexachloro-6-carboxyfluorescein (HEX), or 4,7,2',7'-tetrachloro-6-carboxyfluorescein (TET); a rhodamine dye such as N,N,N,N-tetramethyl-6-carboxyrhodamine (TAMRA) or 6-carboxy-X-rhodamine (ROX); or with a compound such as JOE, RG6, RG110 or Cy5.

19. A method as claimed in any one of claims 14 to 18, wherein the fluorescent label is attached to the 5' end of the telomerase substrate primer.

20. A kit as claimed in any one of claims 14 to 19, wherein each
5 deoxyribonucleotide is present in an amount of from 10 to 100 μ M.

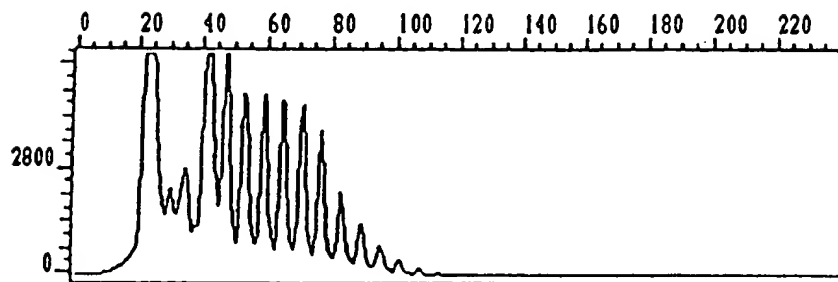
21. A kit as claimed in any one of claims 14 to 20, wherein the PCR primer has the sequence:

10 5'-(CCCTTA)₃CCCTAA-3'.

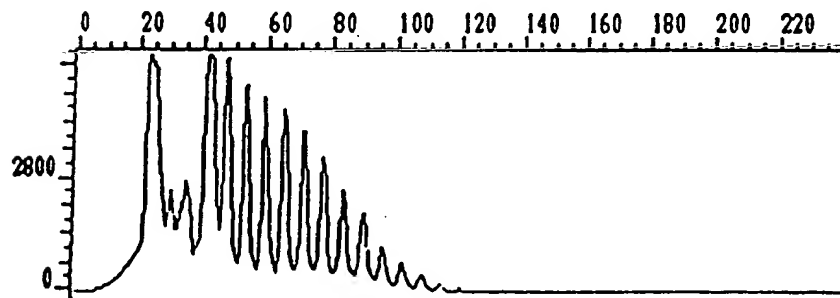
22. A kit as claimed in any one of claims 14 to 21, wherein the PCR primer is present in an amount of 0.05 to 1 μ M.

1/5

MOLECULAR WEIGHT

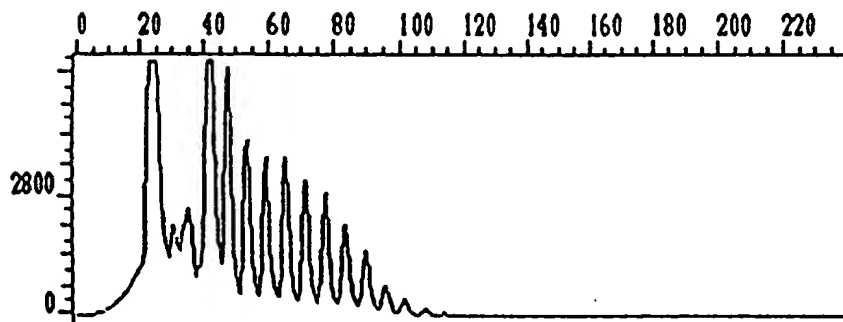


128

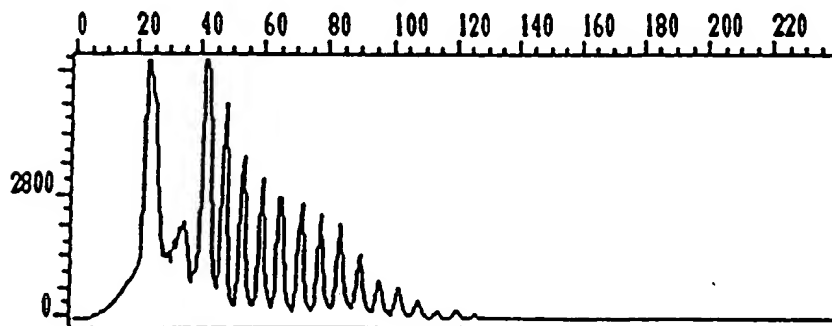


64

PEAK HEIGHT



32



16

FIG. 1(I)

2/5

MOLECULAR WEIGHT

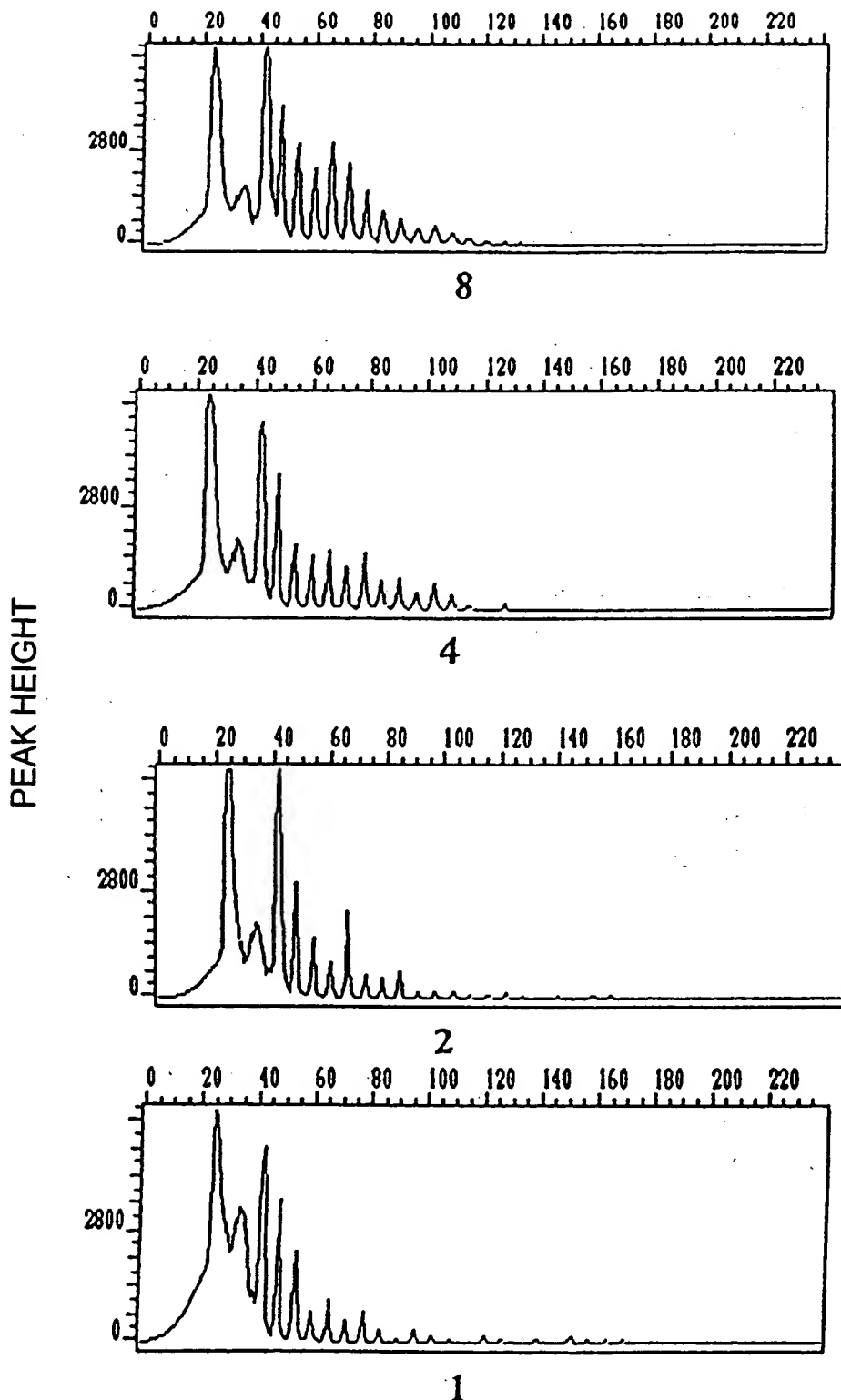
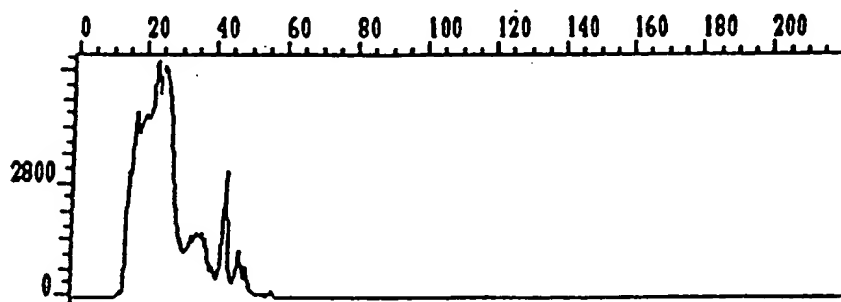
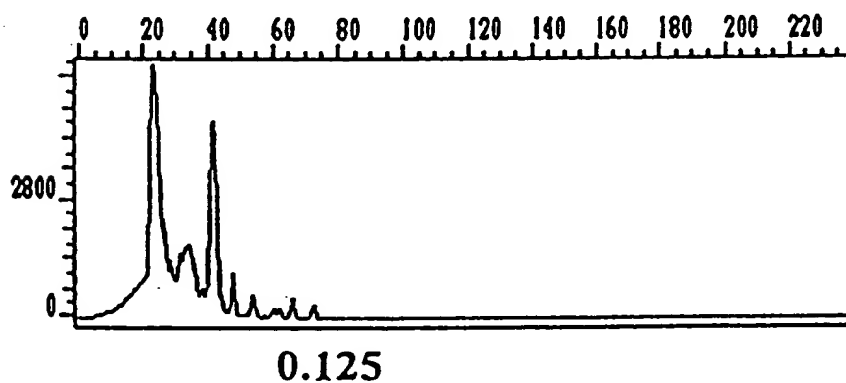
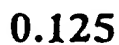
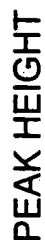


FIG. 1(II)

SUBSTITUTE SHEET (RULE 26)

MOLECULAR WEIGHT



RNASE A+128

FIG. 1(III)

SUBSTITUTE SHEET (RULE 26)

4/5

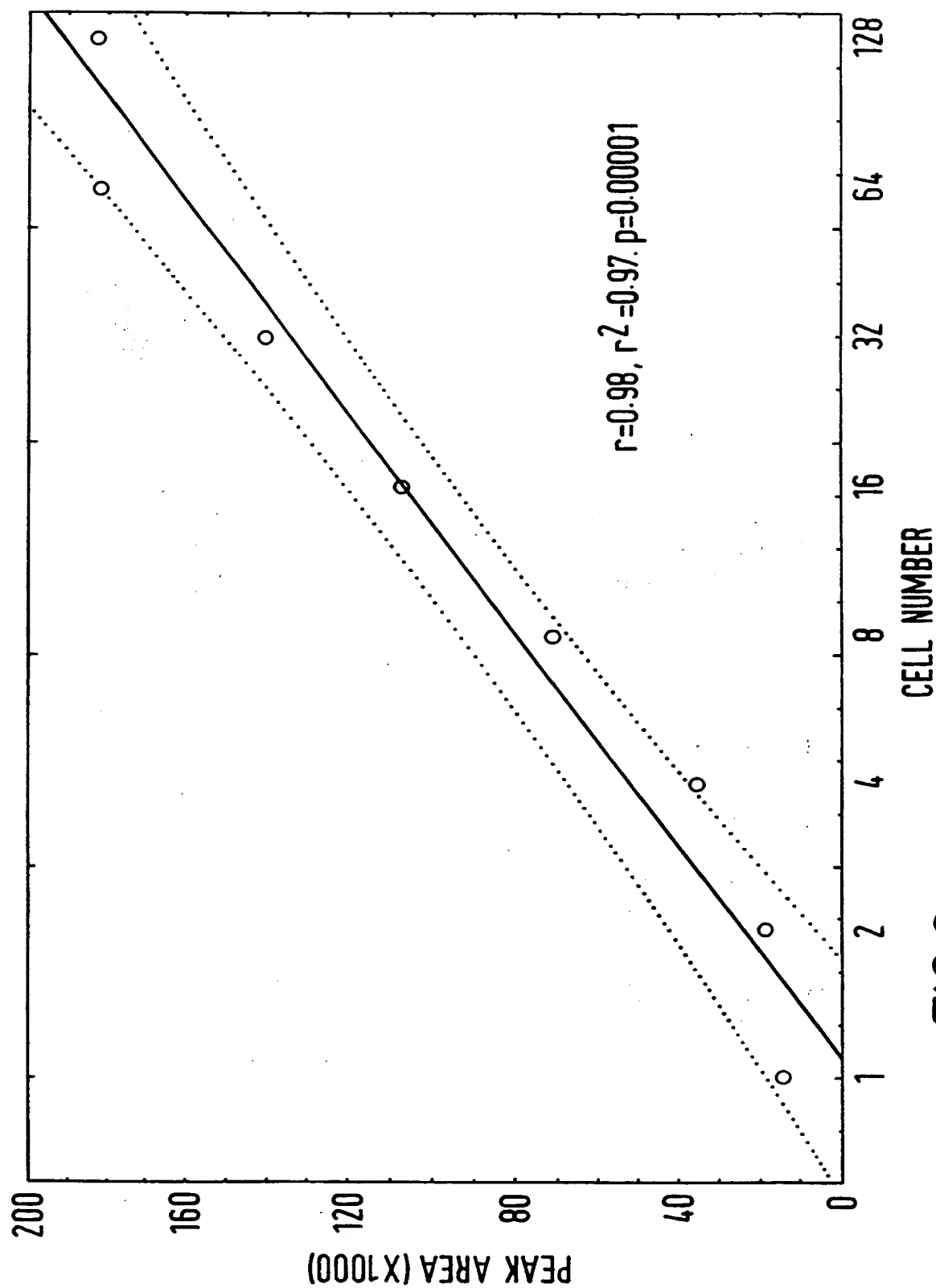


FIG.2

5/5

CCRF-CEM
CEM/VLB₁₀₀
H9
JURKAT
DAUDI
RAJI
RAMOS
KG1A
U937
K562
L929
CEM/VLE₁₀₀
+RNASEA
NEGATIVE
CONTROL

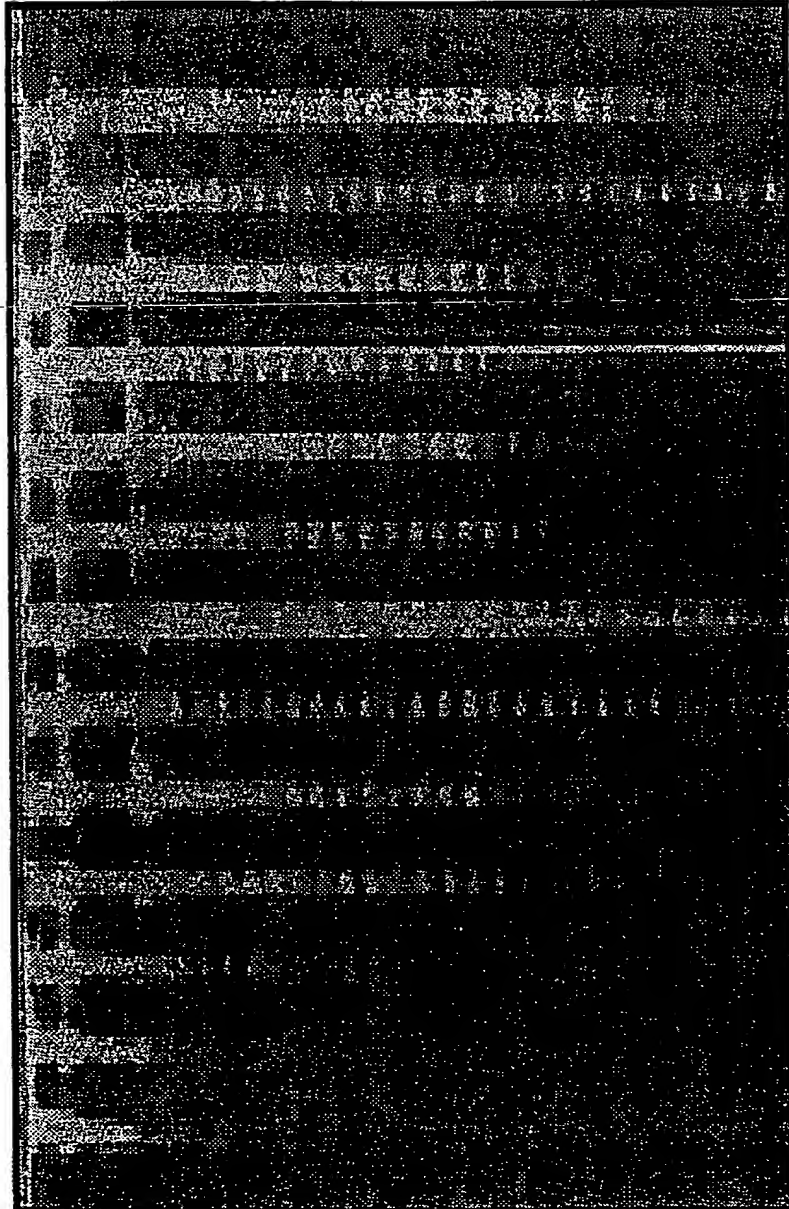


FIG. 3

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 98/02461

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	OHYASHIKI ET AL.: "NON-RADIOISOTOPIC AND SEMI-QUANTITATIVE PROCEDURE FOR TERMINAL REPEAT AMPLIFICATION PROTOCOL" JPN. J. CANCER RESEARCH, vol. 87, no. 4, 1996, pages 329-331, XP000647899 see the whole document	1-22
X	WO 97 15687 A (KIM NAM WOO ;GERON CORP (US); HARLEY CALVIN B (US); WEINRICH SCOTT) 1 May 1997 See example 4, page 51-58 (especially page 55, line 11-15) and Seq.ID 3 and 4 see the whole document --- -/--	1-22

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

17 December 1998

Date of mailing of the international search report

13/01/1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Hagenmaier, S

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 98/02461

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DE 196 44 302 A (BOEHRINGER MANNHEIM GMBH) 5 June 1997 See page 3, line 21-31; claims 34 and 39; and Seq. ID 1 and 7. see the whole document ---	1-22
Y	WRIGHT W E ET AL: "MODIFICATIONS OF A TELOMERIC REPEAT AMPLIFICATION PROTOCOL (TRAP) RESULT IN INCREASED RELIABILITY, LINEARITY AND SENSITIVITY" NUCLEIC ACIDS RESEARCH, vol. 23, no. 18, 25 September 1995, page 3794/3795 XP002028669 cited in the application see the whole document ---	1-22
Y	KIM N W ET AL: "SPECIFIC ASSOCIATION OF HUMAN TELOMERASE ACTIVITY WITH IMMORTAL CELLS AND CANCER" SCIENCE, vol. 266, 23 December 1994, pages 2011-2015, XP002028668 cited in the application see the whole document ---	1-22
Y	"The world of pharmacia biotech '95 '96" 1995 , PHARMACIA BIOTECH , SWEDEN XP002087769 See "Oligonucleotide Synthesis", page 289, Custom synthesis Service and page 295. ---	1-22
P,X	EP 0 811 693 A (KYOWA HAKKO KOGYO KK) 10 December 1997 see the whole document -----	1-22

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 98/02461

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9715687 A	01-05-1997	US 5837453 A US 5804380 A AU 6380896 A	17-11-1998 08-09-1998 15-05-1998
DE 19644302 A	05-06-1997	AU 2623197 A CA 2237823 A WO 9720069 A EP 0863999 A NO 980699 A	19-06-1997 05-06-1997 05-06-1997 16-09-1998 19-02-1998
EP 0811693 A	10-12-1997	JP 10052300 A CA 2206694 A	24-02-1998 03-12-1998